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PRINCIPAL INVESTIGATOR: Ramandeep Rattan, PhD

CONTRACTING ORGANIZATION: Henry Ford Health System
Detroit, MI 48202

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Ramandeep Rattan Phd

rrattan1@hfhs.org
E-Mail:

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14. ABSTRACT

.Ovarian cancer is the leading cause of death from gynecologic malignancies in the United States. Given the ambigcerning the etiology, difficulties in early detection, absence of any markers, low survival rates and the overall obscurity related wrian cancer, more research is needed to identify factors and approaches that could improve ovarian cancer disease initiatgression and disease free survival. One of these factors which have been largely unexplored is the influence of diet and the metabe of the patients. Unfortunately, the role of dietary factors in ovarian cancer prognosis is largely unknown. Particularly, no attenbeen given to the metabolic state of a cancer cell and how this state can be modulated by calorie restriction. In our present worke shown that mice on high energy diet gain a significant amount of weight which is also reflected in higher levels of glucose, insuin and insulin growth factor (IGF-1) compared to mice on regular or 30% calorie restricted diet. The IGF-Insulin pathway has bwn to play a major role in ovarian cancer progression and contributes to the metabolic syndrome. The calorie restricted group er levels of leptin and IGF-1while higher levels of adiponectin. All together, our data shows that the type of diet significantly alters els of physiological growth factors that may influence the growth of tumor.

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Table of Contents

Page

INTRODUCTION

Ovarian cancer is the leading cause of death from gynecologic malignancies in the United States (1). Given the ambiguity concerning the etiology, difficulties in early detection, absence of any markers, low survival rates and the overall obscurity related with ovarian cancer, more research is needed to identify factors and approaches that could improve ovarian cancer disease initiation, progression and disease free survival.

One of these factors which have been largely unexplored is the influence of diet and the metabolic state of the patients. Unfortunately, the role of dietary factors in ovarian cancer prognosis is largely unknown. There are no conclusive guidelines regarding ovarian cancer and food/nutrient intake or physical activity or obesity. There are conflicting reports on this topic that needs further evaluation (2-4). Particularly, no attention has been given to the metabolic state of a cancer cell and how this state can be modulated by calorie restriction. One of the main culprits of metabolic dysfunction is the IGF-Insulin pathway that has been shown to play a major role in ovarian cancer progression and contributes to the metabolic syndrome. Increased levels of insulin and insulin growth factor signaling and increased pro-inflammatory mediators are found in ovarian cancer too. In general, obesity and high caloric intake are known as exacerbating factors in the progression of various cancers including breast and endometrial cancer, while calorie restriction is an amelio rating factor. However, no relationship has been established between diet, energy metabolism and ovarian cancer. Recently some studies have suggested that low-fat dietary pattern may reduce the incidence of ovarian cancer. High energy and calorie restricted diet affects the overall energy metabolism in body which impacts and in turn gets modulated by AMP-activated protein kinase (AMPK).

AMPK is a highly conserved hetero-trimeric serine/threonine protein kinase that acts as an ultra-sensitive cellular energy sensor maintaining the energy balance within the cell (5). Recent studies have identified AMPK activation as having an anti-proliferative effect on cells via regulation of mainly protein translational (mTOR) and lipid biosynthetic pathways to restrict cell growth (5).

This study is designed to understand the role of energy metabolism using nature of diet (high vs low energy) focusing on AMPK as a central energy regulator in ovarian cancer progression using a syngenic mice model. We are proposing a novel **hypothesis** that *low calorie diet will activate AMPK and thereby shifting the energy balance leading to improved outcome in patients with ovarian cancer.* To explore this hypothesis, we had proposed the following specific aims.

Aim 1: To investigate if diet modulation influences ovarian cancer progression via AMPK in C57B6 mouse model using ID8 mouse ovarian epithelial cancer cells.

Under this aim, we were to establish mouse models fed with high energy (HED) and calorie restricted diet (CRD) and examine the modulation of energy metabolism by measuring levels of insulin, adipokines and IGF1 along with oxygen consumption, respiratory quotient, fat oxidation and carboxyhydrate oxidation. After standardization, mice fed with HED and /or CRD will be intraperitoneally injected with ID8-luci cells ($5x10^6$). Effect of dietary energy balance manipulation on mouse ovarian cancer progression will be monitored *in situ* by bioluminescence imaging on days 20, 40 and 60. Following the evaluation of tumor burden, tumor tissue will be processed for IHC and proteomics, microarray and metabolomics studies.

Aim 2: **Therapeutic potential of AMPK activators as diet supplement to enhance energy metabolism in regulating metabolic derangement of tumor progression.**

Under this aim, we will examine the effect of AMPK activators on energy metabolism and ovarian cancer outcome. Mice groups from HED and CRD will be im planted with ovarian tumors as before and treated with metformin (100mg/kg body weight) and berberine (5mg/kg body weight) by oral administration and intraperit oneal, respectively. Post 30 days of treatment, all groups will be processed to examine oxygen cons umption, respiratory quotient, fat oxidation and carboxyhydrate oxidation by using gas anal yzer. Survival of the groups will be determined and tumor progression will be assessed in situ by bioluminescence imaging weekly post ID8-luci injections. Mice will be sacrificed and subjected to proteomic and metabolomic analysis.

The goal of this study is to evaluate a link between nature of food intake (energy-wise) and ovarian cancer which may address how dietary interventions may affect energy metabolism and modulate ovarian cancer outcome.

Due to the change of institute fo r the PI and time required to se t a new lab from scratch, the work is currently behind the propos ed SOW. In the present report we present the standardization of the diet models and details of the tumor implantation experiment in progress.

We and will be working at out complete capacity to make up for the lost time.

BODY OF WORK

TIME LINE OF EVENTS:

The grant was awarded to the PI, Ramandeep Rattan, while at Mayo Clinic, Rochester MN 55905 in 2010. By the time all the paperwork involved was completed the PI had moved to Henry Ford Health System (HFHS), Detroit, MI 48202 in September of 2011. The grant was transferred to HFHS and all the paperwork and other conditions required had to be redone.

1. PI joined HFHS on September 1, 2011.

2. Grant was transferred to HFHS on September 30, 2011

3. The required IACUC protocol was approved from the HFHS IACUC committee on Febuaray 22, 2012. The delay was as we missed the October review date, our protocol could be discussed only in the November meeting. Then due to issues of absentee lay person reviewer in the committee, it lagged for another month and the final approval was given to us in February.

4. After which the ACURO form was submitted. The ACURO approval came on March 19, 2012

5. Ordered custom made High fat, calorie restricted and regular diet from BioServ end of March, 2012, and arrived end of April 2012.

6. The first group of 8 weeks was carried out from June 4- July 31st 2012.

7. The second experiment was started on August 22nd and the mice were injected with tumors on the 19th of October, 2012.

DATA:

1. Establishment of mouse models fed with high energy (HED) and calorie restricted diet (CRD)*:* Female 6 week old C57BL/6 (n=30) mice were randomized (n=10/group) into following three dietary treatment groups:
Group1: regular diet (RD) fed ad libitum
Group 2: fed high energy diet (HED; 60% more fat calories than RD) fed ad libitum
Group 3: Calorie restricted diet (CRD 30% less calories overall than RD)
Mice were weighed weekly. Post 8 weeks mice were euthanized and blood collected for estimation of glucose, insulin and IGF levels among others. The different diets were custom designed by the Bio-Serv Company (NJ, USA). We could not perform the proposed oxygen consumption, respiratory quotient, fat oxidation and carboxyhydrate oxidation, as we were not able to find an instrument where we could perform these studies. Presented in **Figure 1** is the average (n=10) weight progression of the mice on various diets. The high energy diet (HED) fed mice showed approximately 25-30 % weight gain at 8 weeks (blue line) while the calorie

restricted diet (CRD) group (or ange line) showed approximately 5 % weight gain at 8 weeks, similar to that of regular diet fed group (RD) (green line).

2. Mouse fed on different diets show different physiological profiles. Serum collected from above mice on different diets was subjec ted to analysis of glucose concentration and ELISAs for Insulin, leptin, adiponectin and insulin gr owth factor-1 (IGF-1), which constitute a group of growth factors implic ated both in metabolic syndrome and cancer, including ovarian cancer. As shown in **Figure 2**A, glucose levels are signifi cantly higher in the HED group compared to the RD and CRD group, while no signifi cant difference was seen between the RD and CRD groups. Similar observations we re seen in levels of insulin (B), leptin (C) and IGF-1 (E), while no significant change was observed in the levels of adiponectin, except in CRD group which showed a small but signifi cant increase (D). Interesting CRD group showed a significant difference from RD in the levels of leptin and IGF-1, while non –significant trends were observed in case of glucose and insulin levels. All t ogether these parameters show that we were successful in establishing our variable –diet-fed -model in mice as reflected by changes in physiological measurements.

3. Growth profile of ovarian tumor in mice fed with high-energy and low energy diets (ongoing experiment). We have started this experiment end of August. The mice were fed as in 1.
Group1: regular diet (RD) fed ad libitum
Group 2: fed high energy diet (HED; 60% more fat calories than RD) fed ad libitum
Group 3: Calorie restricted diet (CRD 30% less calories overall than RD)

After 30 days on the respective diets, mice were injected with 5 million cells in 200ul of volume by intra-peritoneal route (Figure 3), at which a slight dip in the weights of all mice was observed. We are currently examining the groups by monitoring their weight and diet intake. At 60 days (or earlier depending on the progression of tumor growth), the mice will be sacrificed, blood will be collected for physiological parameters and tumor burden will be assessed. Tumor tissue will then be processed for metabolic and RNA- based arrays.

FIGURE 1

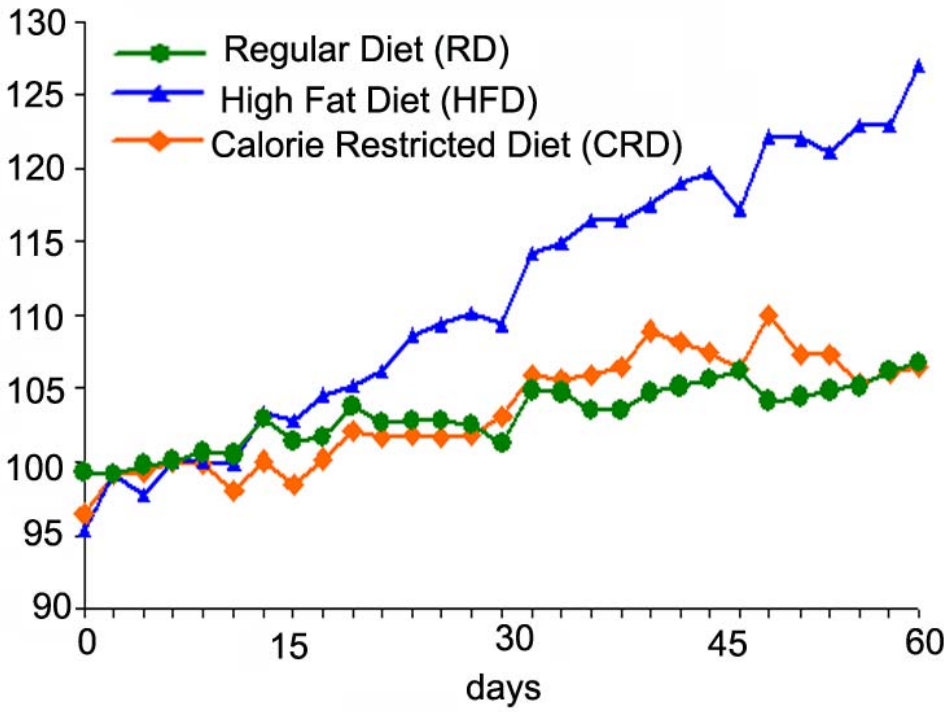

Figure 1: Weight Progression in mice on various energy diets: C57B6 mice (N=10) were fed with High fat (energy) diet (HFH, blue line) or regular diet (RD, green line) or 30% calorie restricted diet (CRD, orange line). Weight of the mice was measured every alternate day until 8 weeks.

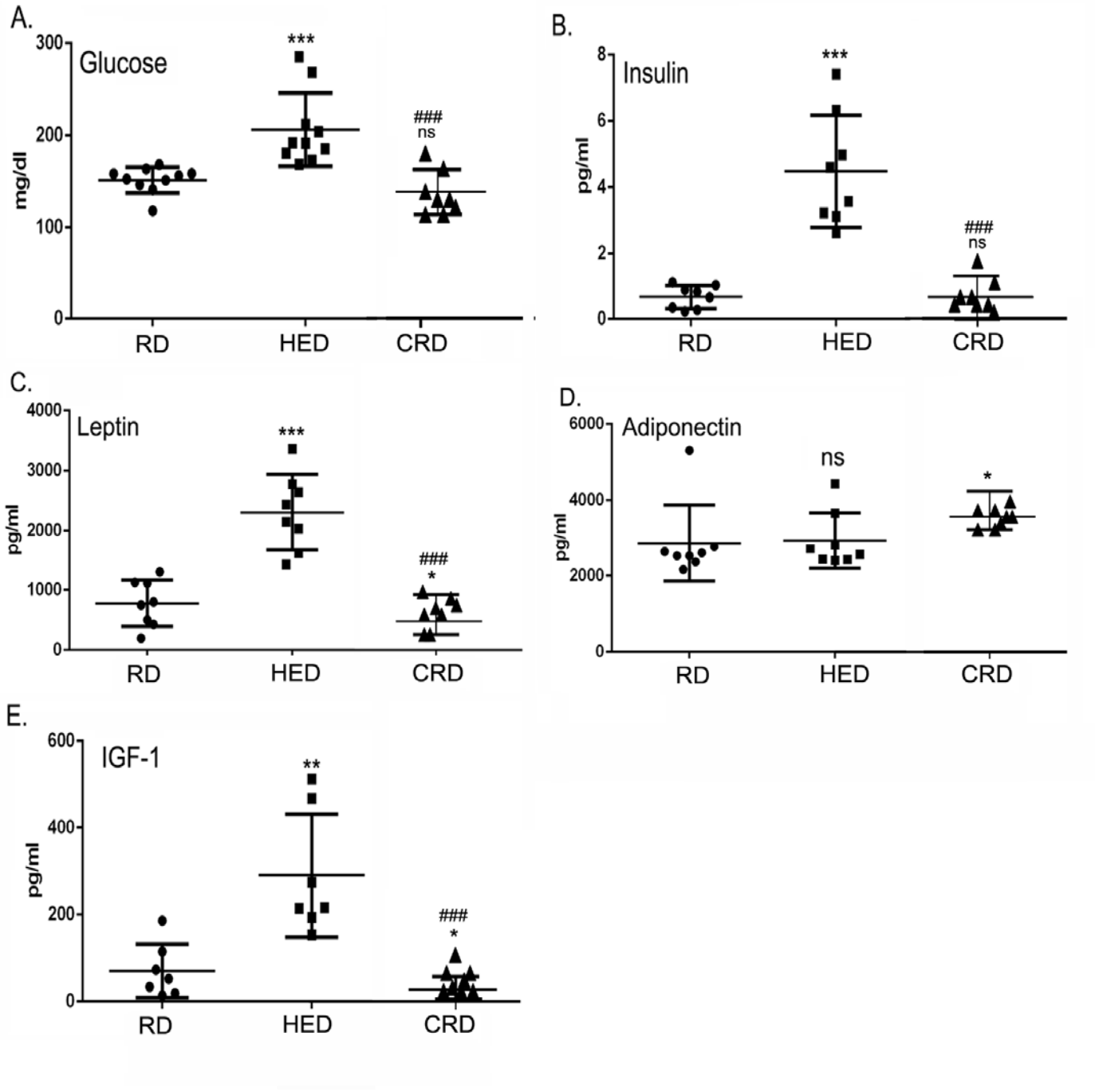

Figure 2: Measure of physiological parameters in mice on regular diet (RD), High energy diet (HED) and Calorie restricted diet (CRD). (A) HED group showed significant higher glucose level (***p<0.001) compared to RD and CRD. The change in CRD mice glucose levels were non-significant compared to RD (ns, ###p<0.001 co mpared to HED). (B) HED group showed significant higher insulin level (***p<0.001) com pared to RD and CRD. The change in CRD mice insulin levels were non-significant compared to RD (ns, ###p<0.001 compared to HED). (C) HED group showed significant higher leptin level (***p<0.001) compared to RD and CRD. The change in CRD mice

glucose levels were significant compared to RD (*p<0.05 compared to RD, ###p<0.001 compared to HED). (D) Adiponectin levels were similar in HED and RD groups. CRD group showed significant increase in adiponectin level (*p<0.05 compared to RD and HED). (E) HED group showed significant higher IGF-1 level (**p<0.01) compared to RD and CRD. The change in CRD mice IGF-1 levels were significant compared to RD (*p<0.05 compared to RD, ###p<0.001 compared to HED).

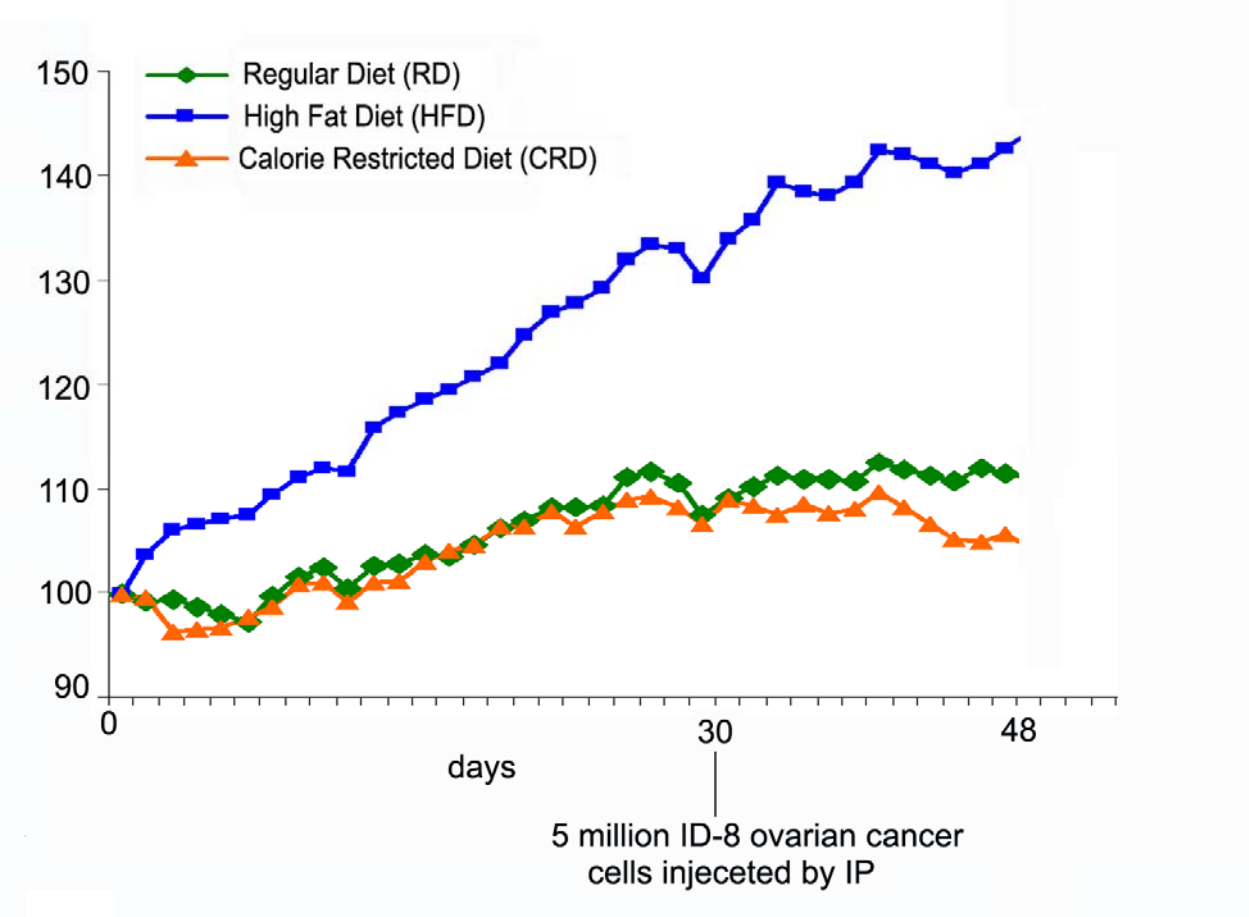

Figure 3: Mouse group progression for Experiment 2.

Key Research Accomplishments:

1. Mice models on various diets (high energy and calorie restriction) has been established. After 8 weeks of being on the respec tive diets, physiological par ameters show significant difference.
2. Mice on high energy diet gain approximately 25% more weight compared to mice on regular diet, while the mice on 30% calorie restricted diet, do not show any significant weight loss compared to regular diet fed mice.
3. Significant high levels were observed in glucose, insulin, leptin and IGF-1, while no significant change was observed in the leve ls of adiponectin between the high energy and regular diet mice.
4. The calorie restricted fed mice showed significant lower levels only in glucose and IGF-1, and slight increase in adiponectin levels; suggesti ng these may play a key role during tumor growth under the influence of various diet patterns.

REPORTABLE OUTCOMES:

1. Mice fed on high energy diet gain that have gained approximately 25% excess weight exhibit higher levels of glucose, insulin, leptin and IGF-1, compared to mice with normal weight and fed on regular diet.
2. Mice fed on 30% calorie restricted fed mice exhibit lower levels only in glucose and IGF-1, suggesting these may play a key role during tumor growth under the influence of various diet patterns.

CONCLUSION

Mice fed on high energy diet gain approximately 25% excess weight and exhibit higher levels of glucose, insulin, leptin and IGF-1, compared to mi ce with normal weight and fed on regular diet. Mice fed on 30% calorie restricted do not show any loss of weight and exhibit lower levels only in glucose and IGF-1, suggesting these may play a key role during tumor growth under the influence of various diet patterns.

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